

Analysis of real samples

The performance of the method was tested with some real water samples. For these studies seven compounds, 5-NH₂-2-NS (**1**), 1-NS (**2**), 3-NH₂-BZS (**3**), 2-NH₂-BZS (**4**), BZS (**5**), 6-NH₂-4-OH-2-NS (**6**) and 6-NH₂-1-OH-3-NS (**7**), were chosen. UV detection for real samples was monitored by using time-scheduled selected wavelengths: 210 nm for compounds **1-5**, and 230 nm for compounds **6** and **7**. The background signals of the matrix compounds were recorded at 230 nm. The identities of the peaks appearing in the real samples were studied by comparing migration times and UV spectra and by further spiking the samples with the reference compounds.

When dealing with real samples, a sample preparation step (*e.g.*, dilution or extraction) is often performed before injection. This is sometimes necessary because real samples often contain substances that increase the conductivity of the matrix, which may interfere with the stacking process. Previous experiments with diluted samples showed that the compounds of interest could be efficiently separated, thus allowing sample stacking with direct injection. Instead of diluting the real samples, we chose to inject lower sample volumes. We decided to inject a sample injection volume of 100 nl for tap and river waters, 60 nl for surface water and 40 nl for WTP waters because for these volumes the analyte response was maximum with no decrease in separation efficiency.

The linearity of the method was checked with tap and Ebro river water samples. Analytical data were calculated in the same way as for Milli-Q water. The linearity of the response was checked in the range between 50 and 250 µg l⁻¹ with determination coefficients higher than 0.994. The LODs was 20 µg l⁻¹. The repeatability and reproducibility of the method were

similar to those for Milli-Q water. Figure 2 shows the electropherograms obtained when unspiked Ebro river water and Ebro river water spiked with $50 \mu\text{g l}^{-1}$ of each compound were analyzed. No NS or BZS peaks were observed in tap and Ebro river water electropherograms and the peak that appeared could not be identified by the UV spectra database.

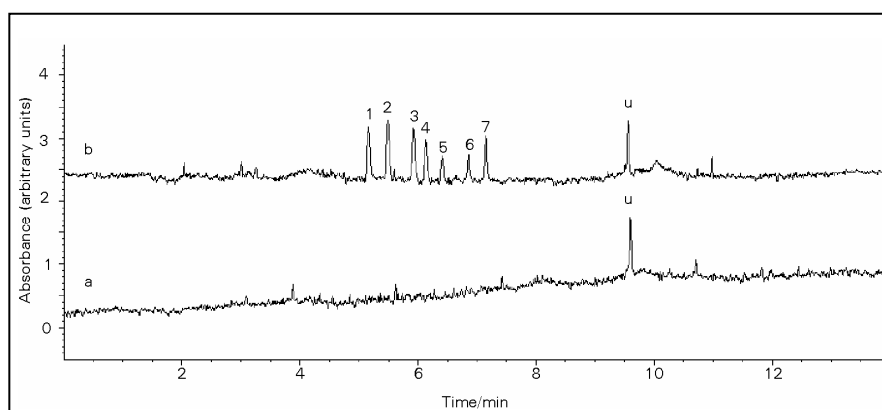


Figure 2 Electropherograms of a river Ebro water sample, (a) unspiked and (b) spiked at $50 \mu\text{g l}^{-1}$ with the seven target compounds after LVSS enrichment procedure. Separations were performed using 20 mM borate buffer at 30 kV and 35 °C. Time-scheduled selected wavelengths. Peak identification: (1) 5-NH₂-2-NS; (2) 1-NS; (3) 3-NH₂-BZS; (4) 2-NH₂-BZS; (5) BZS; (6) 6-NH₂-4-OH-2-NS; (7) 6-NH₂-1-OH-3-NS.

Once the method had been validated, it was tested by analysing real samples with a high content of organic matter: a surface water sample collected near a chemical plant and inflow/outflow water samples from a WTP.

Figure 3 (a) shows the unspiked surface water electropherogram. Two peaks appeared at the migration times for 5-NH₂-2-NS (1) and 1-NS (2), but only the spectrum of the first peak confirmed that it corresponded to 5-NH₂-2-NS. The other peaks that appear in the electropherogram could not be

identified. These results were also confirmed by spiking the surface water sample with $250 \mu\text{g l}^{-1}$ of 5-NH₂-2-NS and 1-NS [Figure 3 (b)]. The response of the 5-NH₂-2-NS was calibrated. This compound was found at a concentration of $104 \mu\text{g l}^{-1}$.

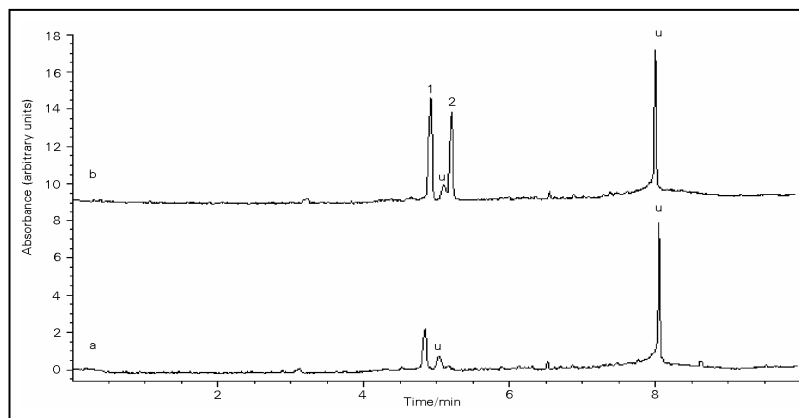


Figure 3 Electropherograms of a surface water sample (a) unspiked, (b) spiked at 0.25 mg l^{-1} with 5-NH₂-2-NS and 1-NS after the LVSS enrichment procedure. Separation conditions and peak identification as in Figure 2.

The method was also applied to evaluate the possible persistence of these compounds in water samples from the inflow and the outflow of a WTP. Figure 4 (a) shows the blank of the inflow WTP water electropherogram overlaid with the spiked sample. Various peaks appeared at the same migration time as the compounds studied. Some of these peaks (A-F) were tentatively identified as aromatic sulfonates by their migration times and by further spiking the samples with the standards. Peaks A and B were tentatively identified as isomeric amino-naphthalenesulfonate (like compound **1**) and isomeric unsubstituted naphthalenesulfonates (like compound **2**), because isomeric naphthalenesulfonates cannot be separated with a simple borate buffer. Peaks C and D were assigned to isomeric amino-benzenesulfonates (like compounds **3** and **4**) and the small peaks E

and F were assigned to isomeric aminohydroxynaphthalenesulfonate (like compounds **6** and **7**). The other peaks that appeared in the electropherogram could not be identified. The response of the unsubstituted naphthalenesulfonate isomer was calibrated. This compound was found at a concentration of $166 \mu\text{g l}^{-1}$. The other peaks could not be quantified because their concentrations were between the detection limit and the quantification limit of the method.

When the outflow WTP waters were analyzed, three of the peaks (C, D and E) which appeared in the inflow WTP water were found. This confirms the persistence of these compounds. Figure 4 (b) shows the blank of the outflow WTP water electropherogram overlaid with the spiked sample. Most peaks appeared in the outflow WTP water at low concentrations and so could not be identified by the UV spectra database. The availability of more selective detection systems such as an UV LIF detector or a MS detector would have enabled the compounds to be confirmed. In order to verify the persistence of these compounds, we compared the peak areas of the compounds tentatively found in the outflow WTP water with those in the inflow WTP water. The peaks were integrated and their normalized peak areas were compared. The normalized peak areas were similar for both samples. The above results are in line with other studies which showed the persistence of some aromatic sulfonate isomers in several real samples.

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In conclusion, this paper has described how an on-line preconcentration technique (LVSS) can be used to determine NSs and BZS by CZE. Stacking can give concentration factors that increase the sensitivity of the method to low $\mu\text{g l}^{-1}$ levels, which is sufficient for some real sample analyses. The combination of stacking, bubble cell capillaries or sensitive cells and

sensitive detectors such as UV LIF detector makes this technique a very promising procedure for on-line sample determination of organic pollutants in real waters at low levels.

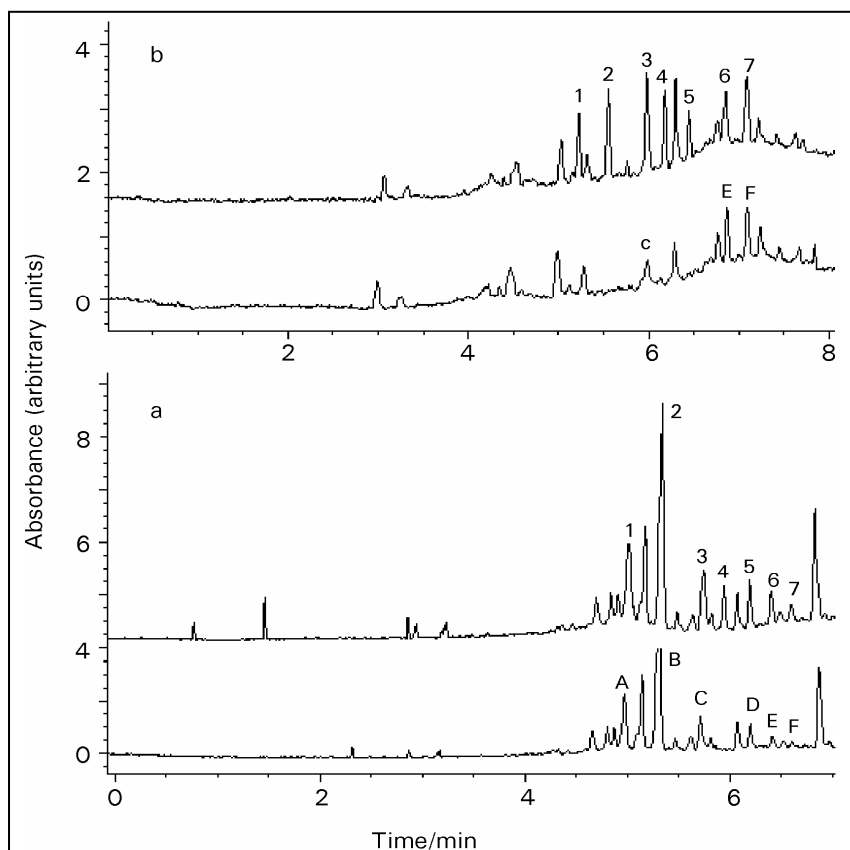


Figure 4 Electropherograms of: (a) blank of the inflow WTP water overlaid with the spiked sample and (b) blank of the outflow WTP water overlaid with the spiked sample. Separation conditions and peak identification as in Figure 2.

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**III.6 PRECONCENTRACIÓ ISOTACOFORÈTICA I DETECCIÓ PER
ESPECTROMETRIA DE MASSES COM A EINES PER MILLORAR LA
DETERMINACIÓ DE COMPOSTOS AROMÀTICS SULFONATS**

Tal com s'ha comentat més amunt, les principals limitacions de l'electroforesi capil·lar quan s'utilitzen els detectors d'absorbància UV-visible són l'escassa sensibilitat i l'ambigua identificació de la presència dels analits en mostres aquoses complexes. En l'estudi anterior es va utilitzar satisfactòriament un sistema de preconcentració *on-capillary* previ a la separació electroforètica per tal de rebaixar els límits de detecció de la tècnica i poder determinar BZS i NS a baixos nivells de concentració en mostres aquoses; malauradament, alguns dels compostos només es van poder identificar per fortificació de les mostres amb els estàndards corresponents ja que als nivells en que es trobaven els analits la identificació dels compostos amb el detector de díodes en fila (DAD) no va ser possible.¹ Això va motivar el desenvolupament d'aquest treball amb un doble objectiu: l'aplicació d'un altre sistema de preconcentració *on-capillary* per tal de millorar els límits de detecció i l'acoblament de la CE a un detector d'espectrometria de masses per tal de millorar la selectivitat de la tècnica.

En aquest treball, igual que en l'anterior, es va seleccionar una barreja de compostos que es poguessin separar per CZE emprant un tampó electrolític senzill. La barreja seleccionada conté els compostos: 2-NS; 5-NH₂-2-NS; 6-NH₂-4-OH-2-NS; 3-NH₂-BZS. La separació es va dur a terme emprant capil·lars de sílice fosa convencionals de 100 o 64.5 cm de longitud (56 cm fins la finestra de detecció) i 75 µm de diàmetre intern, en funció de què l'equip d'electroforesi capil·lar estigués connectat o no al detector d'espectrometria de masses. Els compostos van ser enregistrats a 213 nm (2-NS; 5-NH₂-2-NS; 3-NH₂-BZS) i a 252 nm (6-NH₂-4-OH-2-NS) en el detector de díodes en fila (DAD); quan es va fer servir l'espectròmetre de masses, les masses enregistrades corresponents als fragments [M-H]⁻ van ser 222, 207, 172 i 238 pel 5-NH₂-2-NS, el 2-NS, el 3-NH₂-BZS i el 6-NH₂-4-OH-2-NS, respectivament.

El sistema de preconcentració *on-capillary* emprat en aquest treball es coneix com isotacoforesi (ITP) i dels diferents procediments que es poden trobar a la bibliografia, el que es va adaptar per aquest estudi està basat en els procediments descrits per *N.J. Reinhoud et al.*² Ambdós processos, preconcentració i separació, es van dur a terme emprant un únic capil·lar i un sistema electrolític discontinu format per un electròlit capdavanter (LE) i un electròlit terminal (TE).

En la primera part d'aquest estudi es va dur a terme la selecció dels electròlits capdavanter (LE) i terminal (TE) així com l'optimització del procés d'ITP. L'acetat amònic (10 mM, pH 9.7) es va emprar com a LE i com a electròlit de separació i la β -alanina (10 mM, pH 9.7) es va emprar com a TE. L'optimització del procediment d'ITP (descriu a continuació) va consistir en l'estudi del volum màxim de mostra que es podia injectar sense perdre eficàcia de separació i del temps òptim per preconcentrar isotacoforèticament els analits.

El procediment d'ITP emprat consta de cinc etapes (*figura III.2*): en la primera s'omple el capil·lar amb el LE; en la segona s'injecta hidrodinàmicament un gran volum de mostra i un volum significativament més petit de TE; en la tercera, mantenint el vial que conté el TE a l'extrem d'entrada del capil·lar, es du a terme el procés de preconcentració aplicant un potencial negatiu (invertint la polaritat de la font) alhora que s'aplica una contrapressió per evitar la pèrdua dels analits; en la quarta etapa s'aplica un potencial negatiu, sense aplicar contrapressió, amb el vial de LE a l'extrem d'entrada del capil·lar per eliminar del capil·lar tant el TE com possibles substàncies interferents presents en la matriu de la mostra; en la cinquena i última etapa es torna a invertir la polaritat de la font i té lloc la separació electroforètica dels analits.

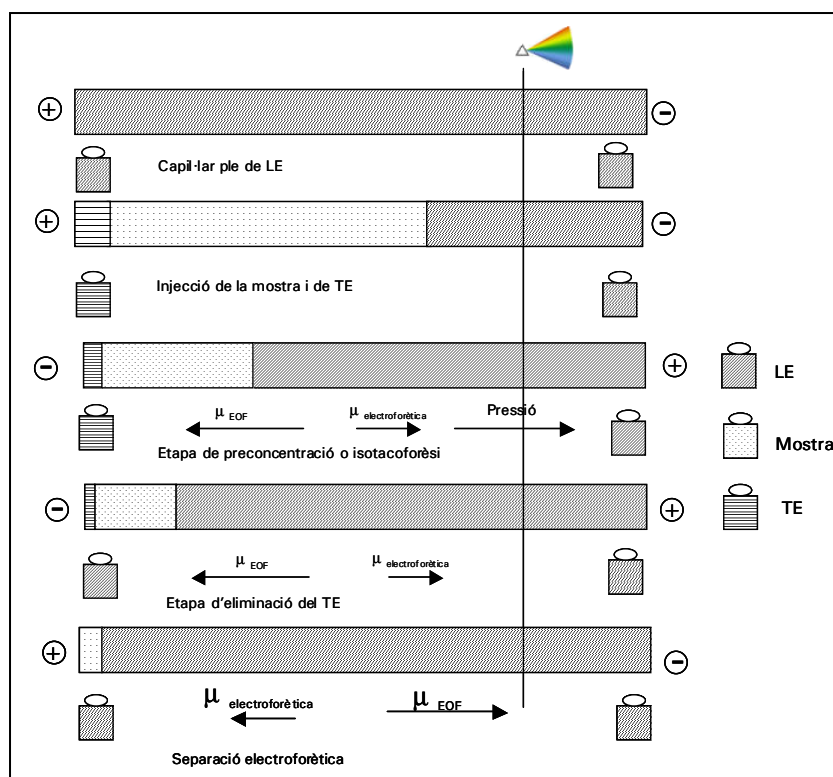


Figura III.2 Esquema del procés de preconcentració i separació que té lloc en ITP-CZE.

En les condicions òptimes de preconcentració (2 ml de mostra injectats i 2.5 min de preconcentració) els límits de detecció van ser de l'ordre de 3-5 $\mu\text{g/l}$, el que va suposar un increment de la sensibilitat d'unes 100 vegades respecte la injecció hidrodinàmica convencional.

En aplicar el procés d'ITP a diverses mostres aquoses –aigua de l'aixeta, aigua de riu i aigües procedents de diverses plantes de tractament d'aigües residuals– va ser necessari introduir una etapa de *clean-up* de la mostra per tal reduir la presència de substàncies iòniques en la matriu de la mostra que interferien en el procés de preconcentració. L'etapa de *clean-up* va consistir

en una extracció en fase sòlida de la mostra emprant un sorbent polimèric altament entrecreuat per tal d'evitar la utilització de parells iònics que poguessin interferir en el procés de separació. Les recuperacions obtingudes van ser superiors al 70% per tots els compostos excepte pel 3-NH₂-BZS que no es va poder recuperar. Encara que el tractament previ de la mostra va millorar significativament el procés d'ITP, es van haver d'adaptar les condicions de preconcentració per cadascuna de les mostres analitzades, augmentant el temps de preconcentració a 7, 10, i 12 min per l'aigua de l'aixeta, de la planta de tractament i del riu, respectivament.

El mètode també es va validar satisfactòriament per a les mostres aquoses analitzades; obtenint-se límits de detecció de l'ordre de 5-15 µg/l. En una de les mostres analitzades procedent d'una planta de tractament d'aigües residuals es va determinar la presència del 2-NS en una concentració de 55 µg/l. En la mostra d'aigua de riu també va aparèixer un pic al mateix temps de migració d'aquest compost, malauradament la coelució d'una substància desconeguda amb l'analit d'interès va impedir la seva identificació amb el DAD.

En la segona part de l'estudi es van optimitzar els paràmetres característics de l'acoblament CE-ESI-MS i es va validar el mètode desenvolupat establint els intervals de linealitat, els límits de detecció i la repetitivitat.

L'espectròmetre de masses es va connectar a la sortida de l'equip d'electroforesi capil·lar emprant una interfície d'electrosprai (ESI) ortogonal per CE-MS (*figura III.2*), treballant en mode negatiu.

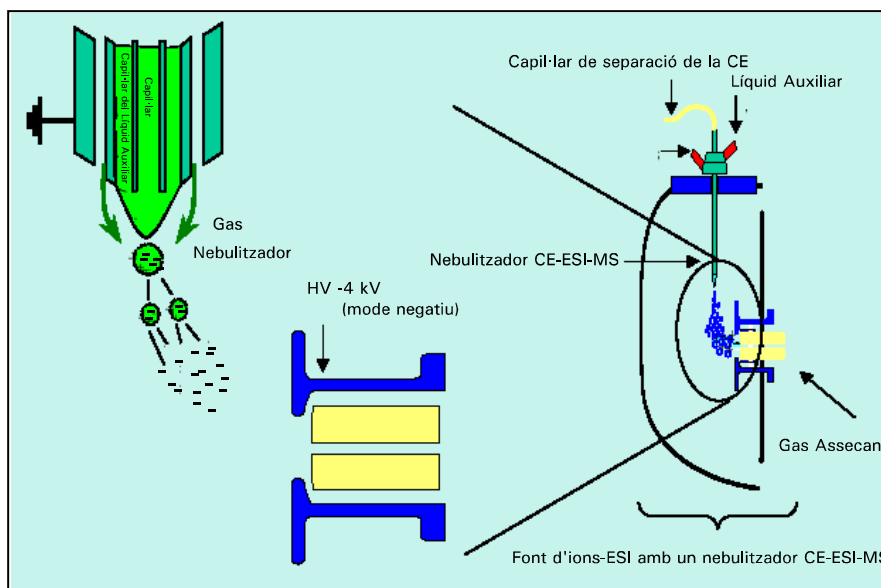


Figura III.2 Interfície CE-ESI-MS

Els paràmetres de la interfície ESI es van optimitzar en *full scan* (m/z 70 a 270) per infusió d'una solució patró de 50 mg/l dels compostos en estudi. Es van escollir com a criteris de selecció dels paràmetres optimitzats, la forma i la resposta del pic, el temps de migració i la intensitat del corrent desenvolupat, tant l'electroforètic com el de l'espectromètre de masses.

Es va seleccionar un voltatge de capil·lar de 3500 V, un fragmentor de 100 V, perquè donava la millor relació fragmentació/sensibilitat i una temperatura de 200° per a la desolvatació de l'aigua. En aquestes condicions el ió més abundant per tots els compostos correspon al $[M-H]^-$.

També es va optimitzar la pressió de nebulització en observar que tant els temps de migració com l'eixamplament dels pics com la sensibilitat es veien fortament afectades per ella. Es va programar el seu valor en el temps per a les diferents etapes del procés: no es va aplicar pressió durant la

injecció de la mostra, es van aplicar 10 p.s.i. durant la separació CZE-DAD i 20 p.s.i. durant la separació CZE-ESI-MS; això va permetre obtenir resultats repetitius amb una bona eficàcia de separació i temps d'anàlisi més curts. El flux de gas assecant, després d'estudiar el seu efecte, es va fixar a 10 l/min.

Un altre paràmetre que va esdevenir molt important en l'optimització del procés va ser la composició i el flux del líquid auxiliar. El que va donar abundàncies més elevades va ser una barreja d'isopropanol-aigua (80:20, v/v) amb un 0.1% d'hidròxid amònic per afavorir el contacte elèctric i va ser subministrat a un flux de 4 µl/min.

Es va intentar reduir el temps d'anàlisi manipulant la pressió, però, tot i obtenir els resultats desitjats es va descartar aquesta possibilitat pels efectes negatius que l'acompanyaven, pèrdua de resolució, de simetria dels pics i de repetitivitat.

El mètode es va validar estudiant l'interval de linealitat, establint els límits de detecció i avaluant-ne la precisió, utilitzant el 2-NS com a compost model. La linealitat del mètode, entre 0.25 i 5 mg/l, va ser satisfactòria, obtenint-se un límit de detecció de 0.1 mg/l.

Posteriorment es va aplicar a l'anàlisi d'una mostra real. En la primera part d'aquest estudi (ITP-CZE) es va sospitar la presència del 2-NS en la mostra de riu analitzada; en la segona part es va tornar a analitzar aquesta mostra emprant el sistema CZE-ESI-MS, la qual cosa va permetre no només confirmar la presència d'aquest compost en la mostra, sinó també poder-lo quantificar (SIM mode) en una concentració de 2.1 mg/l.

Els estudis realitzats en aquest treball, dels quals s'adjunta una còpia a continuació, han estat enviats a la revista *Electrophoresis* per a la seva publicació.

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**ISOTACHOPHORETIC FOCUSING AND MASS SPECTROMETRY DETECTION
AS TOOLS FOR IMPROVING THE DETERMINATION OF AROMATIC
SULFONATES IN CAPILLARY ELECTROPHORESIS**

SUMMARY

We explored isotachopheresis–capillary zone electrophoresis with diode array detection on a single capillary to find out how to increase the injection volume and decrease the detection limits of aromatic sulfonates in capillary zone electrophoresis. The isotachopheretic focusing was performed by applying a negative voltage in conjunction with hydrodynamic backpressure programming, and the terminating buffer was removed before the capillary zone electrophoresis separation, which resulted in highly sensitive determinations. The isotachopheretic focusing increased the signal response of conventional hydrodynamic injection by a factor of 100, whereas the separation efficiency was unaffected. The limits of detection of the method were between 3 and 5 $\mu\text{g l}^{-1}$. The method was successfully used to determine these compounds in water samples. Experimental conditions for capillary electrophoresis–mass spectrometry were optimised and applied to determine aromatic sulfonates in water samples. These techniques enables the 2-naphthalenesulfonate to be determined in water samples.

Keywords: Aromatic sulfonates; Isotachopheretic focusing; Electrospray ionization-mass spectrometry; Water samples.

INTRODUCTION

The importance of capillary electrophoresis (CE) as an analytical tool has increased significantly over the last decade. Nowadays, the major challenges of using CE separations to analyse environmental samples are to obtain suitable determination limits and to unambiguously identify pollutants in complex matrices.

Although sensitivity is still one of the main limitations of CE, there are several means of solving this problem.^{1,2} One is to use laser induced fluorescence or phosphorescence detection methods.^{3,4} This approach, however, requires analytes to have certain characteristics for detection.

Another approach is to preconcentrate the sample before the CE separation using conventional off-line sample preconcentration procedures such as solid phase extraction (SPE).² A third approach is to increase the sample loadability by on-line electrophoretic concentration.⁵⁻¹³ The electrophoretic concentration methods increase the sample loadability without losing separation efficiency and can be performed using continuous (stacking procedures) or discontinuous (isotachophoretic procedures) electrolytes. Although there are significant differences between these procedures, one common feature is that the analytes are concentrated at the boundary between the sample and the buffer/s over which there is a difference in the electrical field strength when a voltage is applied.⁵⁻¹³

There are two different configurations for performing isotachophoresis-capillary zone electrophoresis (ITP-CZE): the single-capillary (one column) or dual-capillary (two columns coupled together) mode.⁷⁻¹³ The main

advantage of the latter configuration is that ITP can be used for sample cleanup and only the zones of interest are then analysed by CZE. The main advantage of the single mode is that it is easily automated and the instrumentation required is simple. In single ITP–CZE, the sample is inserted between a leading electrolyte (LE) whose ionic mobility was higher than that of the sample and a terminating electrolyte (TE) whose ionic mobility was lower. All analytes with ionic mobilities between the LE and the TE are then focused into discrete zones and migrate isotachophoretically to the border of the LE.^{7,8} After ITP focusing, the CZE separation takes place.

The combination of CE with mass spectrometry (MS) provides highly selective detection of compounds in various complex mixtures.¹⁴⁻¹⁶ Of the different types of CE-MS interfaces that have been described in the literature since CE-MS coupling was first reported by Olivares et al.,¹⁷ electrospray ionization (ESI) has become the most popular method for coupling CE with MS.¹⁸ ESI is a soft ionization technique that sprays and ionizes analytes directly from the solution phase to the gas phase. However, CE-ESI-MS is still not considered to be a fully developed technique, mainly due to current technical and chemical limitations. While the outlet of an HPLC system can be connected quite easily to the electrospray inlet, CZE-ESI interfacing has other drawbacks: the high voltage of the separation capillary, the high voltage of the MS instrument and the low flow rates. Moreover, while volatile buffers in HPLC are well established and compatible with MS, most of the buffers in CE are chosen for their separation selectivity and are non volatile. Not only do non volatile high-saline buffers run the risk of significantly decreasing the ion intensity (through ion-pairing effects or the prevention of ion evaporation during the electrospray process), but salt crystals can also contaminate or even plug the MS entrance.

Aromatic sulfonate (AS) isomers like benzene- (BZS) and naphthalenesulfonates (NS) are widely used in industrial and domestic processes.¹⁹⁻²² Although they are not very toxic and have no genotoxic or carcinogenic effects, the microbiological persistence of some compounds is a potential environmental risk. They are usually present at low levels in environmental samples so sensitive and selective electrophoretic methods are required if they are to be detected.^{3,4,6,14,19,22}

As far as we know, ITP-CZE has not been used to determine AS in water samples and CE-MS has scarcely been used for identifying such compounds in water samples.¹⁴ Therefore, the aim of the present paper was twofold: to overcome not only the lack of sensitivity of absorbance detection by applying an on-capillary isotachophoretic enrichment procedure but also the difficulty in unambiguously identifying AS in water samples by coupling CE to ESI-MS. Four aromatic sulfonates (5-amino-2-naphthalenesulfonate, 5-NH₂-2-NS; 2-naphthalenesulfonate, 2-NS; 3-amino-benzenesulfonate, 3-NH₂-2-BZS and 6-amino-4-hydroxy-2-naphthalenesulfonate, 6-NH₂-4-OH-2-NS) were selected as model compounds for performing ITP-CZE and CE-ESI-MS.

MATERIALS AND METHODS

Chemicals and standards

Standards of the four aromatic sulfonates were purchased from Aldrich (Beerse, Belgium) and Fluka (Buchs, Switzerland). A stock standard solution of 1000 mg l⁻¹ of each compound was prepared in water (several drops of NaOH were usually added to enhance solubility) and stored in a dark-glass flask at 4 °C. Working solutions were prepared daily by diluting the standard

solutions with water (Milli-Q or water samples). β -alanine was purchased from Sigma (St. Louis, USA) and ammonium acetate was purchased from Aldrich. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, USA). The leading/background electrolyte was 10 mM ammonium acetate and the terminating electrolyte was 10 mM β -alanine. They were both adjusted to pH 9.7 with ammonia. The leading and the terminating electrolytes were prepared daily, de-gassed by ultrasonication and filtered through a 0.20 μm nylon syringe filter (Tracer, Barcelona, Spain) before use. The sheath liquid was also filtered through a 0.45 μm nylon membrane filter (Whatman, Maidstone, England) and de-gassed by ultrasonication before use.

Equipment

A Hewlett Packard (Palo Alto, USA) $^{3\text{D}}$ CE system equipped with a UV diode array detector was used. On-capillary absorbance detection took place at 56 cm from the anodic end. The compounds were recorded at 213 nm (5-NH₂-2-NS, 2-NS and 3-NH₂-BZS) and 252 nm (6-NH₂-4-OH-2-NS). Electropherograms were recorded using the HP Chemstation chromatographic data system.

A mass spectrometer was connected to the exit to the CE-DAD system through an electrospray ionisation (ESI) interface. The CE column inlet was placed at the same height as the CE-ESI-MS probe to prevent siphoning. The ESI-MS measurements were carried out in the negative ionization mode and performed in a single quadrupole HP series 1100 MSD (Hewlett-Packard, Palo Alto, USA).

Procedures

Before each session, the capillary was conditioned by flushing for 5 min with 0.1 M NaOH and for 15 min each with water and leading electrolyte. At high sensitivity determinations, precautions were taken to prevent carry over effects. This means that the capillary was flushed between runs, that the electrolytes were refreshed after each analysis and that blank analyses were made between series of analyses. Using this procedure, it was found that the run-to-run reproducibility was maintained.

The ITP-CZE and the CZE-MS experiments were carried out in 64.5-100 cm \times 75 μ m I.D. fused silica capillaries (eCAP™ Capillary Tubing, Beckman, USA), respectively. To maintain a stable electrospray, a 20-mm portion of the polyimide coating was removed from the outlet end of the capillary. This procedure improved the reproducibility characteristics at the probe tip. The capillaries were thermostatted using a Peltier system at 25°C and the power supply was operated in the constant voltage mode at 30 kV or 25 kV for ITP-CZE and CZE-MS experiments, respectively.

The mass spectrometer was operated in negative ion mode by applying a voltage of 3500 V to the capillary. The gas temperature was set at 200°C and the fragmentor parameter was set at 100 V. Nitrogen was used as the drying gas with a flow-rate of 10 l min⁻¹ and as the nebulizer gas with a pressure of 20 p.s.i. Coaxial sheath liquid, consisting of isopropanol-water (80:20, v/v) in the presence of 0.1% ammonia, was delivered at 4 μ l/min by a Hewlett-Packard 1100 Series binary pump equipped with a 1:100 flow splitter. Mass spectra in full-scan mode were collected by scanning in the range 70-270 *m/z*. In selected ion monitoring (SIM) acquisition, the most abundant ions in each compound were monitored. The main ions obtained

for each compound, corresponding to the fragments $[M - H]^-$, were 222, 207, 172 and 238 for 5-NH₂-2-NS, 2-NS, 3-NH₂-BZS and 6-NH₂-4-OH-2-NS, respectively.

The ITP-CZE procedure was performed as follows: (i) The capillary was rinsed with LE using the flush mode (at a pressure of 1000 mbar approx.); (ii) The injection took place hydrodynamically at a pressure of 50 mbar, and then a small plug of TE (1% of the injected sample volume) was also hydrodynamically injected at the same pressure; (iii) The analyte focusing was carried out by applying a voltage (-30 kV) in conjunction with a hydrodynamic backpressure (50 mbar) with the TE vial at the inlet (anodic) capillary end and the LE vial at the outlet capillary end. The focusing step lasted between 0.8-2.5 min or 7-12 min, for standards and water samples respectively, depending on the injected zone length and the sample matrix composition, (iv) For the terminating buffer zone to be removed from the capillary by ITP, the TE vial is replaced by a vial containing the LE and a voltage of -30 kV was applied without hydrodynamic pressure. The current was monitored so that the moment to switch from the ITP to the CZE mode could be precisely timed. When the voltage was constant the current increased as long as the terminating zone length decreased. The CZE equipment was programmed so that the voltage stopped at a defined current threshold (about 95% of the current generated when the capillary is completely filled with LE). This step usually took less than 1 min; (v) When the sample zone was approaching the capillary inlet the CZE run started.

Sample preparation

Tap water, river water and water from several waste water treatment plants (WWTP) were analysed.

Samples were collected in dark glass bottles and stored at 4°C. They were filtered through a 0.45 µm nylon membrane filter (Whatman, Maidstone, England) before use. Tap, river and WWTP water samples were also passed through an Oasis HLB solid-phase extraction (SPE) cartridge for sample clean-up. The clean-up step was carried out using a solid-phase extraction manifold connected to a vacuum pump (Teknokroma, Barcelona, Spain). The Oasis HLB SPE cartridges were preconditioned with 7 ml of methanol followed by 3 ml of Milli-Q water (pH 3). The sample (20 ml) was passed through the cartridge and the aromatic sulfonates were eluted with 3 ml of methanol and 1 ml of Milli-Q water.

RESULTS AND DISCUSSION

Optimisation of the ITP-CZE-DAD method

We selected the appropriate leading and terminating electrolytes on the basis of data from the literature [7,8,10,13] and after we had carried out several experiments using sodium tetraborate, sodium phosphate and ammonium acetate as LE and β-alanine and cacodylic acid as TE. We selected ammonium acetate as LE and background electrolyte (BGE) because of its volatile characteristics and the satisfying separations when it was used as buffer with CZE. The LE/BGE pH value was set at 9.7 for an optimum CZE separation. β-alanine was selected as TE, and its pH was set at the same value as that of the LE in order to avoid pH gradient contributions. The mobility difference between ammonium acetate and β-alanine at that pH was large enough to form an isotachophoretic window where all compounds undergo focusing.

In order to determine low levels of AS in water samples, we studied how to lower the detection limits with an isotachopheretic focusing procedure. The injection volume and the focusing step of this procedure were optimised and the moment at which ITP was switched to CZE was adjusted.

The injection volume was optimised with a standard solution of $50 \mu\text{g l}^{-1}$ of each aromatic sulfonate. The relationship between peak area and injection volume was linear with injection volumes between 0.5 and 2.0 μl (15-70% of the total capillary volume). Figure 1 shows how injection volume affects the analysis of a standard solution and also that the capillary can be filled to 70% without band broadening. When larger volumes were injected into the capillary, the peak shapes of the four AS became asymmetric, as has already been observed and attributed to capillary overloading and the mismatch of mobilities between the analytes and coions.^{10,11}

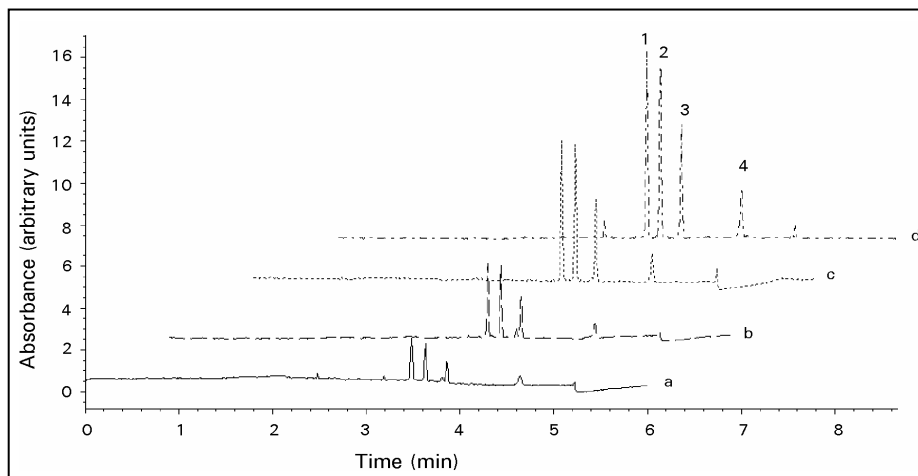


Figure 1 Effect of the injection volume on the isotachopherograms of a standard AS mixture of $50 \mu\text{g l}^{-1}$. Injected volume: 15% (a), 25% (b), 50% (c), and 70% of the total capillary volume (d). CZE conditions: running buffer 10 mM ammonium acetate, pH 9.7; applied voltage, 30 kV; temperature 25°C. Peak identification: 5-NH₂-2-NS (1), 2-NS (2), 3-NH₂-BZS (3) and 6-NH₂-2-NS (4).

During the focusing step (step (iii) in 2.3 procedures), the negative voltage (30 kV) led to an electroosmotic flow in the direction of the capillary inlet, so a hydrodynamic backpressure (50 mbar) was applied to prevent the analytes from migrating out of the capillary.¹⁰ The focusing step was considered to be complete when all ions moved isotachophoretically under steady state conditions.²³ In agreement with other authors,^{10,24} we found that the focussing step depended on the volume of the sample injected. At larger injection volumes longer focusing times were necessary. The focusing time for a standard solution containing 50 µg l⁻¹ of each compounds increased from 0.8 min for an injection volume of 0.5 µl to 2.5 min for 2.0 µl. The effect of focusing time on performance in ITP-CZE is important because shorter times decrease the signal because focusing is incomplete and this could affect the resolution of the separation.

The current was monitored so that the moment for automatically switching from ITP to CZE could be determined. It was adjusted to prevent the analytes from leaving the capillary and to minimise the TE plug remaining in the capillary, the purpose of which was to prevent the homogeneity of the electrical field from being disturbed. Without it the efficiency and migration times of the CZE run could be affected. The CZE equipment was programmed so that at a defined threshold of current the ITP automatically switched to CZE. A compromise was reached between the effects at lower and higher values and this point was set at 95% of the current generated when the capillary is completely full of LE. When the current at the moment of the switching zone is lower than 93% broadening occurs, and when it is higher than 97% there is a slight but significant decrease in the response of the compounds eluted first.

The analysis for the ITP-CZE run (2.0 μl injection volume) took only 8 min. This included a flush of 1.5 min with leading buffer. Flushing between analyses was necessary for migration times to be reproducible. The analyte responses obtained by ITP-CZE were 100 times greater than those obtained by conventional CZE.

Validation of the ITP-CZE-DAD method

Once the loadability had been optimized, we studied the linearity and reproducibility of the ITP-CZE method in the range 10-1250 $\mu\text{g l}^{-1}$ for the four aromatic sulfonates. All correlation coefficients were higher than 0.999. The detection limits, based on a signal-to-noise ratio of 3, were 3 $\mu\text{g l}^{-1}$ for 5-NH₂-NS, 2-NS, and 3-NH₂-BZS, and 5 $\mu\text{g l}^{-1}$ for 6-NH₂-4-OH-2-NS.

The reproducibility between days in ITP-CZE was studied for a standard solution containing 10 $\mu\text{g l}^{-1}$ of each compound. The relative standard deviations (RSD, $n=5$) were lower than 1.2% and 3.5% for migration times and corrected peak areas, respectively.

The performance of the method was then checked with some water samples such as tap water, river water and WWTP water samples. The presence of high concentrations of matrix constituents in the sample can prevent the ITP steady state from being reached.²⁵ One way of overcoming the incompatibility of the sample matrix with the ITP conditions is to pretreat the sample by removing most of ionic matrix constituents. An additional sample pretreatment usually improves reproducibility, selectivity and ITP focusing time of ITP-CZE.¹⁰

After several experiments which confirmed the incompatibility of our sample matrices with the ITP conditions, a clean-up step was carried out before the ITP-CZE procedure. Because of the difficulty of retaining highly polar analytes such as AS, several highly crosslinked polystyrene-divinylbenzene polymeric SPE cartridges, LiChrolut EN, Isolute Env +, and Oasis HLB were tested. There were no significant differences between the recoveries obtained with the tested polymeric sorbents for most of the AS. Oasis HLB was selected for performing the clean-up step because it gave slightly higher recoveries for the amino-hydroxy derivative. Unfortunately, the amino-benzene compound was nearly lost because of its high solubility in water, which reduces its retention in all the SPE cartridges tested. The breakthrough volumes of the AS were determined by extracting different sample volumes (10 and 50 ml) of Milli-Q water. The samples were spiked at different concentrations to obtain the same theoretical final amount. 20 ml was selected because at higher volumes recoveries decreased. When 20 ml of Milli-Q water spiked with 0.2 mg l⁻¹ of each compound were extracted, recoveries were higher than 70% and RSD values (n=3) were between 7-12%. The recoveries for tap and WWTP water samples were similar to those specified for Milli-Q water.

Although the clean-up step improved the performance of ITP-CZE — as reported in the literature ¹⁰ — the ITP focusing time had to be adjusted for each sample. The times were 7 min, 10 min and 12 min for tap water, WWTP water and river water sample, respectively.

When a tap water sample was analysed, no significant peak eluted at the same migration time as the analytes being studied. When tap water samples spiked with different levels of analytes were analysed by ITP-CZE, linearity was good between 30 and 1000 µg l⁻¹. The correlation coefficients were

between 0.991 and 0.998 for 6-NH₂-4-OH-2-NS and 5-NH₂-2-NS, respectively. The detection limits (LOD) and quantification limits (LOQ), calculated as three times and ten times the signal-to-noise ratio, were 5 and 15 µg l⁻¹ for 5-NH₂-2-NS and 2-NS, and 10 and 30 µg l⁻¹ for 6-NH₂-4-OH-2-NS, respectively. Comparing ITP-CZE to conventional CZE for tap water samples, the increases in areas were similar to those with Milli-Q water samples. Figure 2 shows the electropherograms (with and without SPE clean-up) and the isotachopherogram obtained when a 1 mg l⁻¹ spiked tap water sample was injected into the capillary. The repeatability of the ITP-CZE method was determined for a tap water sample spiked with 100 µg l⁻¹ of each compound. The RSDs (n=3) were lower than 3% and 7% for migration times and peak areas, respectively. For reproducibility between days (n=3) values were lower than 5% and 10%, respectively.

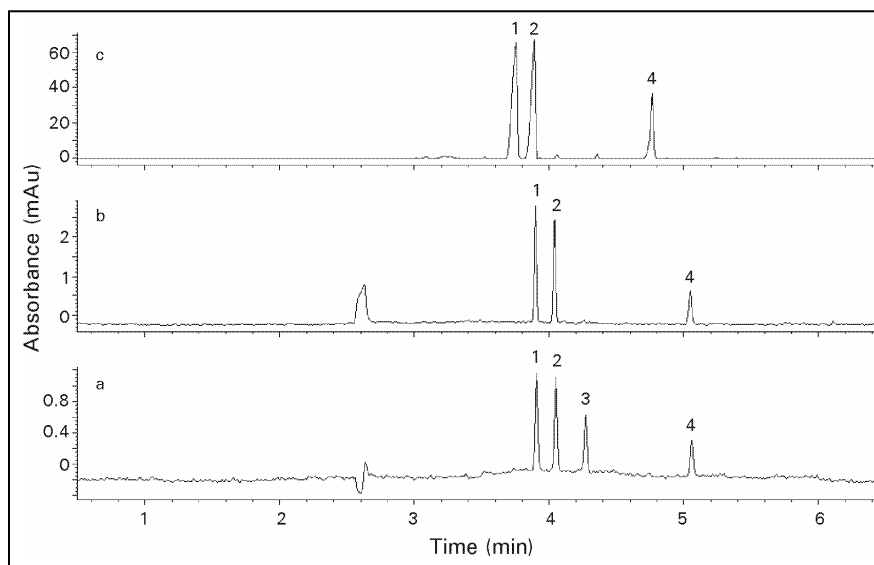


Figure 2 Electropherograms obtained when a tap-water sample spiked with 1 mg l⁻¹ of each AS was hydrodynamically injected (a) without the SPE clean-up; (b) with the SPE clean-up using an Oasis HLB cartridge; and (c) with the SPE clean-up and then isotachophoretically focused. The CZE conditions are the same as those reported in Fig.1. Peak identification as in Figure 1.

Several WWTP water samples from different sites were analysed. The recoveries and the linearity for these samples were similar to those specified above for tap water. 1-NS and 2-NS cannot be separated by CZE nor identified by MS. Therefore, we shall refer to 2-NS in the text because 2-NS is the one that is most frequently found in environmental waters. Figure 3 shows that in one of the samples a peak appeared at the same time as the isomeric compound 2-NS. It was tentatively identified as 2-NS by comparing its spectra with that of the standard with a match factor of 996. It was quantified at $55 \mu\text{g l}^{-1}$ level.

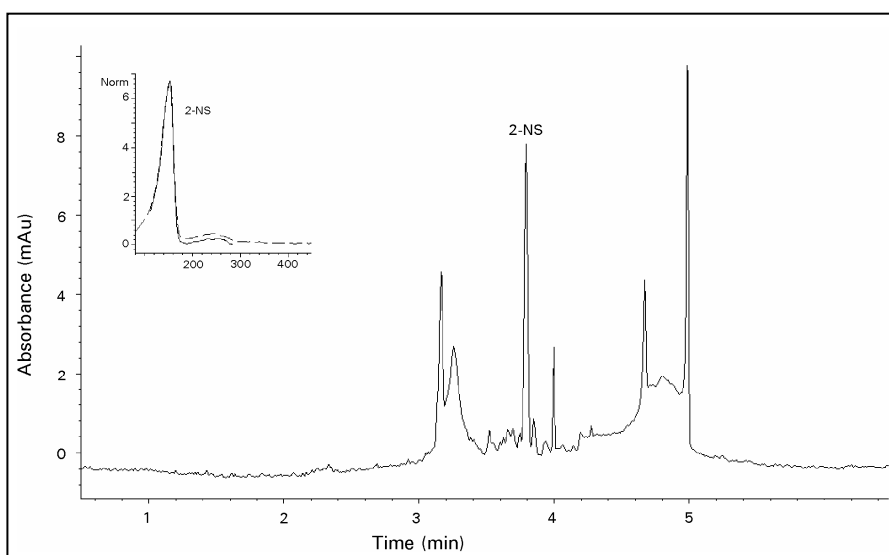


Figure 3 ITP-CZE-DAD electropherogram of a river water sample. The insert shows the overlay of the spectra of the peak and that of the standard (2-NS). The CZE conditions are the same as those reported in Figure 1.

When analysing a river water sample, also appeared a peak at the same migration time as 2-NS. The presence of such compounds in the sample was suspected by the comparison between the spectra of the peak and that of the standard (match factor lower than 990); and the study of the peak

purity spectra revealed the coelution of some unknown substance with the analyte of interest.

Currently, the most efficient way of overcoming the ambiguous identification of these analytes when they are analysed by CE is to use more selective detectors such as mass spectrometry.

CZE-ESI-MS

In order to test the capacity of mass spectrometry as a detection system for capillary electrophoresis, the experimental parameters were optimised first. The criteria for evaluating the optimum parameters were the shape and response of the peak, the migration times and the intensities of the electrophoretic and electrospray current.

ESI parameters were optimized in the full scan mode (scanning from m/z 70 to 270) by infusing 50 mg l^{-1} of a standard solution of AS through the CE capillary at a pressure of 50 mbar. The probe voltage was set at 3500 V. The fragmentor was optimised between 50-200 V. It was set at 100 V as a compromise between sensitivity and fragmentation. In these conditions the most abundant ion for all the compounds is $[M - H]^-$.

A temperature of 200°C was suitable for water desolvation. It was noted that migration times, sensitivity and peak broadening were significantly affected by the nebulizing pressure. Therefore, after several experiments, the nebulizing pressure was operated by using a time-scheduled table. It was set at 0 p.s.i. during the hydrodynamic injection, at 10 p.s.i. for the CZE-DAD separation and at 20 p.s.i. for the CZE-ESI-MS separation. In agreement with other authors we did not set the nebulizing pressure during

the hydrodynamic injection because it negatively affected both the response and the migration time of the peaks.¹⁵ The drying gas flow rate was set at 10 l min⁻¹.

It has been reported that the sheath liquid is critical to the performance of the CZE-ESI interface. Therefore, we optimised its composition by varying the type (methanol, isopropanol and acetonitrile) and the percentage (20-90%) of organic solvent. Because the negative mode was used, ammonia was added to the sheath liquid to facilitate the electric contact. We tested values between 0.1 and 0.5% of ammonia and since we found no significant differences, we selected 0.1%. 2-NS was used as the reference compound. Figure 4 shows that an isopropanol–water mixture (80:20, v/v) in the presence of 0.1% ammonia results in the highest ion abundance signal.

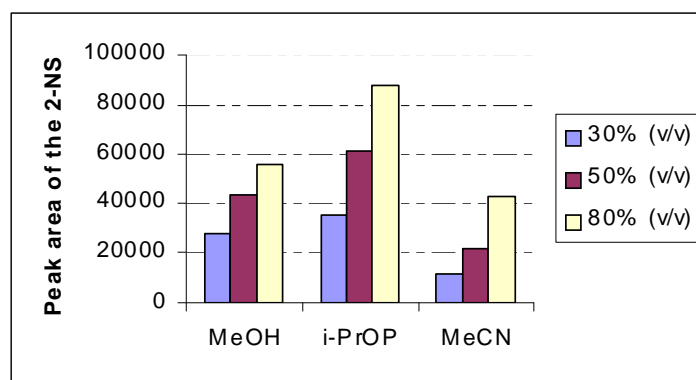


Figure 4 Effect of the type and the percentage of organic solvent in the composition of the sheath liquid. Studies were performed in the presence of 0.1% (v/v) ammonia.

The sheath flow was expected to dilute the CE sample zone as it passed concentrically around the CE column effluent and mixed with it. This effect

was investigated by varying the sheath flow rate and measuring the signal in the TIC, as the 2-NS ion ($m/z = 207$) eluted. Because only sheath flow rate values of 2-8 $\mu\text{l}/\text{min}$ produced a stable electrospray and no significant differences were observed over the range studied, 4 $\mu\text{l}/\text{min}$ was selected as optimum in terms of signal response and stability.

In the CZE-ESI-MS experiments, the DAD detector was used to check the migration time of the analytes, and thus determine the absence of critical retroelution. We also examined the effect of applying an additional slight overpressure to reduce the analysis time or a back pressure to compensate for the effect of retroelution produced by the nebulizing pressure. Although we observed the expected effects in both cases, the resolution, peak shape and reproducibility of the separation was worse. Applying pressure might be used to an advantage in some situations,¹⁵ but it did not improve the quality of the separation in this case.

Figure 5 shows the total ion current (TIC) and the extracted ion current (EIC) electropherograms of a 50 mg l^{-1} AS standard solution. In order to evaluate the sensitivity of the method, an AS standard solution was injected into the CE capillary in varying amounts while the mass spectrometer was operated in the selected ion monitoring (SIM) mode. In SIM acquisition, the most abundant ion of each compound ($[\text{M-H}]^+$) was monitored. Using 2-NS as the model compound, the method was found to be linear throughout the 0.25–5 mg l^{-1} concentration range, with a correlation coefficient of 0.997. The limit of detection for 2-NS under the SIM mode was 0.1 mg l^{-1} . The detection sensitivity of CE-MS (SIM) was similar to that of CE-DAD. The repeatabilities, expressed as RSD ($n=3$), of the migration time and peak area were found to be about 3.5% and 10% respectively.

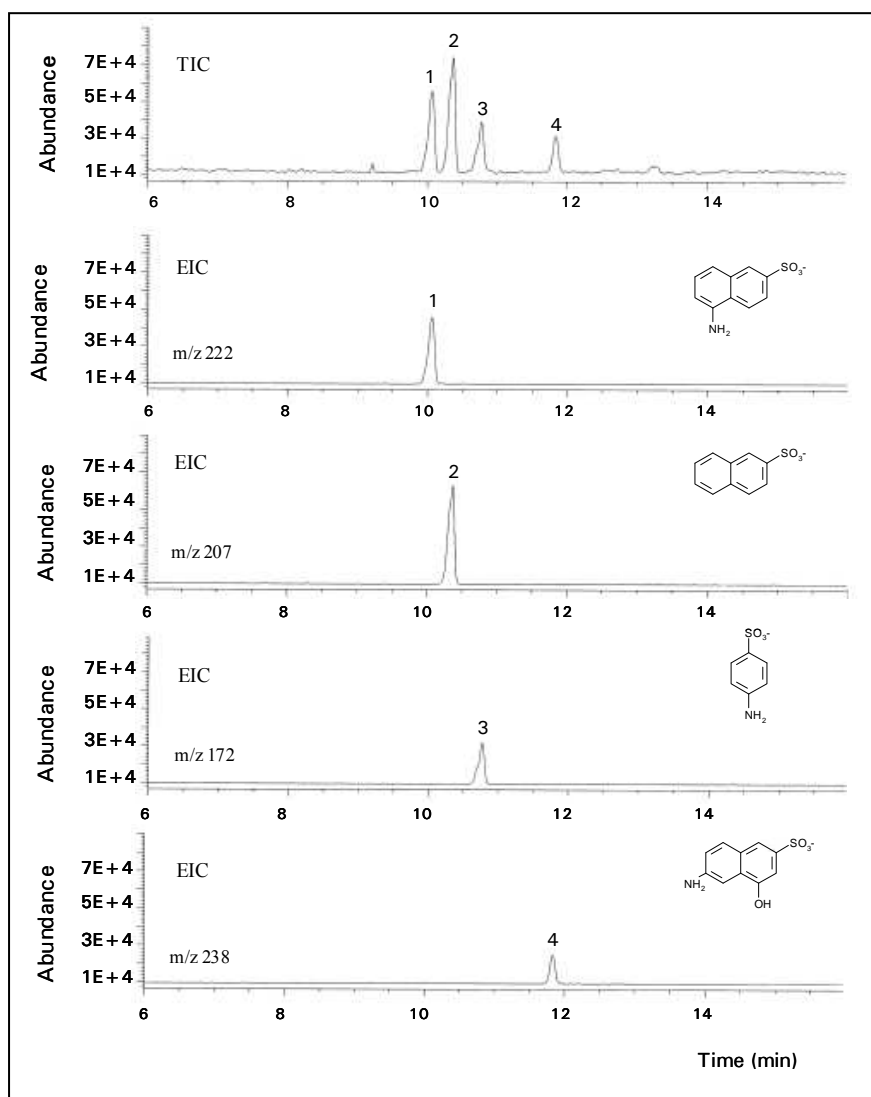


Figure 5 CE-ESI-MS total ion current (TIC) and extracted ion current (EIC) profiles for the base peak of the aromatic sulfonates ($50 \mu\text{g l}^{-1}$). Peak identification as in Figure 1. For conditions, see text.

When the river water sample was analysed using the DAD detector, we suspected the presence of 2-NS (Figure 6a) so we analysed it again using the MS detector. The analysis by CZE-ESI-MS produced a total ion current (TIC) electropherogram with two peaks (Figure 6b).

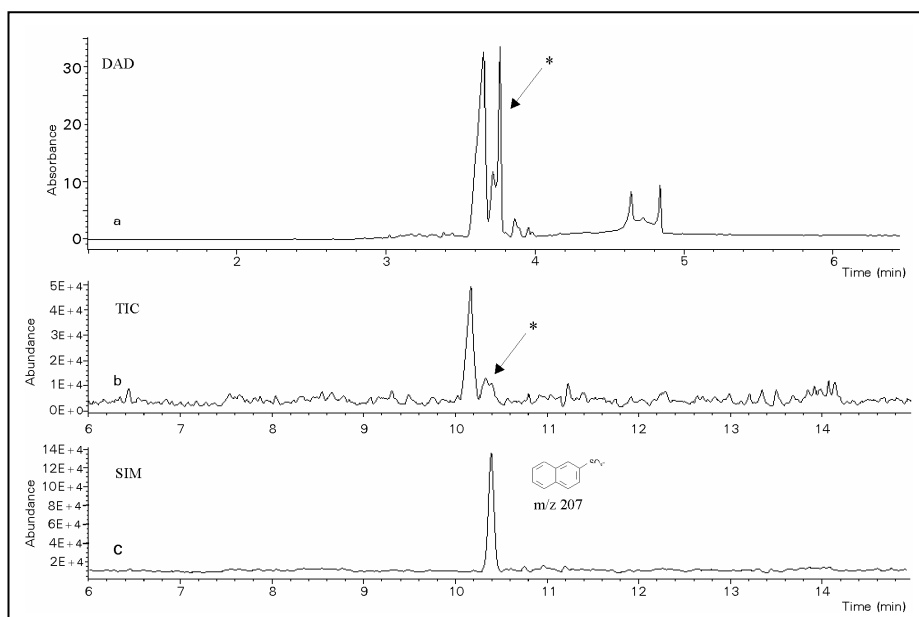


Figure 6 ITP-CZE-DAD electropherogram (a) and full scan total ion current, TIC, (b) and selected ion monitoring, SIM, (c) CZE-ESI-MS electropherograms of a river water sample containing 2-NS. For conditions, see text.

The spectra from those peaks were examined and appeared to contain the expected molecular ion of 207 m/z characteristic of 2-NS. The extracted ion current (EIC) electropherogram at 207 m/z gave a single peak at 10.5 minutes corresponding to this compound. The peak was also quantified in SIM acquisition (Figure 6c). The concentration was 2.1 mg l^{-1} with a standard deviation value of 15% ($n=3$). CE-MS cannot distinguish between the isomeric sulfonates 1-NS and 2-NS, because they cannot be separated by CZE and they have the same main ion (207 m/z). The other peaks that

appeared in the electropherogram could not be identified. When lower level concentrations of aromatic sulfonates are present in water samples, an enrichment procedure such as an isotachophoretic preconcentration should be used to lower the detection limits.

CONCLUSIONS

The results of the present paper clearly show for the first time that ITP focusing in a single-capillary mode is a suitable on-line enrichment procedure for aromatic sulfonates. The results also show that ITP-CZE can be applied to determine aromatic sulfonates in water samples. When water samples were analysed a SPE clean-up step was performed before the analysis to eliminate undesirable compounds from the sample matrix. The ITP method described is fully automated, reproducible and linear at low concentrations. A CZE-ESI-MS method was optimised, which enabled aromatic sulfonates to be identified and quantified in water samples. Mass spectrometry detection increases confidence in the identification of the compounds in water samples.

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CAPÍTOL IV

PERSPECTIVES FUTURES

Pel que fa als compostos en estudi, tal com s'ha exposat en el recull bibliogràfic sobre la seva determinació (capítol II, secció II.2) i en la part experimental (capítol III) de la present tesi, hi ha diversos mètodes que poden ser satisfactòriament emprats per separar-los mitjançant tècniques electroforètiques amb unes condicions d'anàlisi senzilles i una eficàcia de separació elevada.

El fet que aquests compostos no estiguin sotmesos a cap regulació, que hi hagi una gran varietat d'isòmers amb mobilitats electroforètiques molt semblants i que els autors utilitzin diferents barreges de compostos per fer els estudis, dificulta que es pugui recomanar una única tècnica electroforètica. Gairebé tots el mètodes descrits a la bibliografia donen bons resultats per separar barreges que continguin diferents compostos (no substituïts i substituïts amb diferents grups) amb sistemes electrolítics senzills. Però, quan en una barreja es tenen diferents isòmers d'un mateix compost, es fa necessari introduir de sistemes micel·lars o afegir ciclodextrines per tal d'aconseguir-ne la separació.

En aquest sentit, encara que són molt escasses les publicacions disponibles a la bibliografia i que per tant és difícil treure'n conclusions clares, tal vegada el desenvolupament de l'electrocromatografia capil·lar permeti resoldre barreges complexes d'aquests compostos ja que combina els avantatges de l'electroforesi capil·lar amb els de la cromatografia de líquids.

Una altra possibilitat és aprofundir en el desenvolupament de nous sistemes de separació, emprant, per exemple, tensioactius més específics, que siguin fàcils de manipular com ara els tensioactius no iònics i que es puguin utilitzar en concentracions més baixes.

Generalment en la determinació d'aquests compostos per CE es fan servir detectors poc selectius com els d'absorbància UV-visible de longitud d'ona fixa o de díodes en fila, i en alguns casos s'empren els detectors de fluorescència que, malgrat que són més selectius, no permeten identificar els compostos d'interès a baixes concentracions. Actualment per resoldre aquest problema es duen a terme nombrosos estudis: alguns autors estan desenvolupant nous sistemes de detecció per fluorescència induïda per làser i de fosforescència amb el doble objectiu de millorar la selectivitat i la sensibilitat de la tècnica en la determinació de compostos aromàtics sulfonats i de simplificar-ne l'ús i rebaixar el cost perquè puguin estar a l'abast de qualsevol laboratori. D'altres autors comencen a utilitzar l'acoblament de l'electroforesi capil·lar a l'espectrometria de masses per tal de millorar la selectivitat de la tècnica.

Per tal de millorar la sensibilitat, en la majoria de treballs disponibles a la bibliografia s'utilitza l'extracció en fase sòlida com a sistema de pretractament i/o preconcentració de les mostres; malauradament té una aplicabilitat limitada a causa de la polaritat dels compostos aromàtics sulfonats. Actualment, l'extracció en fase sòlida amb addició de parells iònics continua sent la que dóna millors recuperacions per a la majoria de compostos aromàtics sulfonats. Aquest sistema d'extracció, però, no és adequat per treballar amb l'electroforesi capil·lar ja que els parells iònics poden modificar l'eficàcia de la separació, això fa que quan es treballa amb aquests sistemes sigui necessari introduir una etapa d'eliminació del parell iònic abans que tingui lloc la separació electroforètica. Per altra banda, l'extracció amb els sorbents polimèrics de darrera generació que poden ser utilitzats per a l'extracció dels compostos d'interès sense l'addició de parells iònics no permet extreure els compostos més polars, amb la qual cosa l'ús és limitat. Per tant, el desenvolupament de nous sorbents més selectius per

aquests tipus de compostos contribuiria a millorar la sensibilitat de l'electroforesi capil·lar i acostar-ne l'ús al de la cromatografia de líquids.

Recentment, diversos autors s'han interessat en l'aplicació de tècniques de preconcentració *on-capillary* per determinar a baixos nivells de concentració compostos aromàtics sulfonats per CE. Aquests sistemes encara no estan totalment desenvolupats per a aquests compostos i presenten certes limitacions, però els resultats obtinguts són prometedors i ofereixen, respecte a l'extracció en fase sòlida, l'important avantatge de permetre la preconcentració de qualsevol compost aromàtic sulfonat, independentment de la presència de determinats grups en la seva estructura. Conseqüentment, tant l'aprofundiment en l'ús d'aquest tipus de procediments, com d'altres (utilització de capil·lars de preconcentració acoblats als de separació, etc.) que s'han aplicat satisfactòriament en altres camps per millorar la sensibilitat de l'electroforesi capil·lar constitueixen un clar camí en l'aplicació d'aquesta tècnica en l'anàlisi mediambiental dels compostos d'interès.

CAPÍTOL V

CONCLUSIONS

Les conclusions derivades del treball realitzat en la present tesi doctoral són les següents:

1. L'electroforesi capil·lar és una tècnica que presenta un gran potencial de separació, com s'ha demostrat en el desenvolupament de diferents mètodes per separar diverses barreges de benzensulfonats i naftalensulfonats.
2. L'electroforesi capil·lar per zones en mode contraelectroosmòtic permet separar barreges complexes de BZS i NS monosulfonats i disulfonats que contenen diversos compostos no substituïts i substituïts amb diferents grups.
3. En aquest tipus de separacions, l'addició de solvents orgànics com l'etanol o l'isopropanol pot contribuir a millorar significativament la separació. Tot i amb això, l'addició d'aquests modificadors provoca un increment força significatiu del temps d'anàlisi que no es pot reduir modificant les condicions de separació (augmentant el voltatge i la temperatura).
4. L'electroforesi capil·lar per zones en mode coelectroosmòtic addicionant a l'electròlit modificadors de flux electroosmòtic com ara l'HDB, permet separar diversos compostos en un temps d'anàlisi significativament més curt.
5. L'electroforesi capil·lar per zones en mode coelectroosmòtic addicionant a l'electròlit modificadors de flux electroosmòtic (HDB o CTAB) i solvents orgànics, possibilita la separació de barreges de BZS i NS amb diversos isòmers d'un mateix compost.

6. La cromatografia micel·lar electrocinètica en mode contraelectroosmòtic amb tensioactius aniònics (SDS) o no iònics (Brij 35) també permet separar barreges de BZS i NS que contenen diversos isòmers d'un mateix compost. Dels diferents tensioactius examinats, el no iònic (Brij 35) és el que dona les millors separacions en temps d'anàlisi més curts.
7. La cromatografia micel·lar electrocinètica en mode coelectroosmòtic amb tensioactius catiònics com ara el CTAB no només contribueix a invertir el flux electroosmòtic sinó que introdueix interaccions addicionals que possibiliten separar isòmers, com ara els naftalendisulfonats no substituïts, que no es poden resoldre mitjançant cap altra de les tècniques electroforètiques aplicades.
8. S'ha demostrat la utilitat del *sample stacking* mitjançant la injecció de grans volums de mostra (LVSS) com a tècnica de preconcentració *on-capillary* per reduir els límits de detecció dels compostos en estudi. L'aplicació va permetre de reduir els límits de detecció d'entre 0.15 i 0.3 mg/l (corresponents a la injecció hidrodinàmica convencional) a 5 i 10 µg/l.
9. També se n'ha demostrat l'aplicabilitat en l'anàlisi de diverses mostres aquoses (d'aixeta, de riu, d'aigua superficial i dels afluents d'entrada i sortida d'una planta de tractament d'aigües residuals). El mètode també va ser validat per algunes d'aquestes mostres (aixeta i riu), amb les quals es van assolir uns límits de detecció lleugerament superiors als obtinguts en aigua Milli-Q, de 20 µg/l, a causa de la influència de la matriu de la mostra en el procés de

preconcentració. Es va identificar i quantificar el 5-NH₂-2-NS (aigua superficial) i el 2-NS (afluent d'entrada a la planta depuradora) en concentracions de 104 µg/l i 166 µg/l, respectivament.

10. L'ús de la isotacoforesi (ITP) com a tècnica de preconcentració *on-capillary* acoblada a la CZE també va permetre millorar considerablement la sensibilitat de l'electroforesi capil·lar, ja que va incrementar en 100 vegades el senyal del detector obtingut mitjançant la injecció hidrodinàmica convencional i assolir límits de detecció d'entre 3 i 5 mg/l.
11. Els diferents sorbents polimèrics altament entrecreuats disponibles comercialment que s'han emprat com a sistema de *clean-up* de les mostres analitzades mitjançant la ITP-CZE, han donat recuperacions superiors al 70% per a diversos naftalensulfonats; malauradament l'amino-benzensulfonat no es va poder recuperar amb cap dels sorbents.
12. La ITP-CZE també ha estat satisfactòriament aplicada a la determinació a baixos nivells de concentració dels compostos en estudi en mostres reals, ja que s'han obtingut uns límits de detecció lleugerament superiors als aconseguits en aigua Milli-Q, d'entre 5 i 10 µg/l, a causa de la influència de la matriu de la mostra en el procés de preconcentració. Es va identificar i quantificar el 2-NS en una concentració de 55 µg/l en un afluent d'una planta de tractament d'aigües residuals.

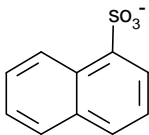
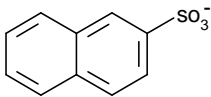
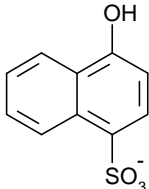
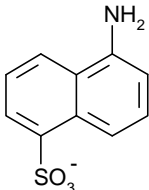
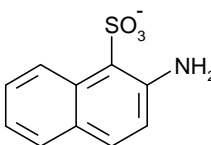
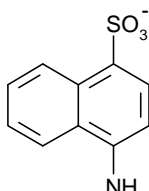
13. S'ha demostrat la possibilitat d'acoblar la l'electroforesi capil·lar a l'espectrometria de masses mitjançant una interfície d'electrosprai per determinar de BZS i NS. També s'ha demostrat la importància de l'optimització de diversos paràmetres experimentals com ara el fragmentor, la pressió del nebulitzador o la composició del líquid auxiliar per a l'acoblament CE-ESI-MS.

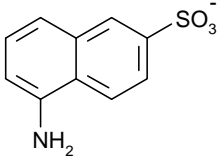
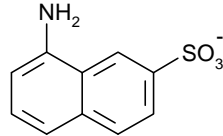
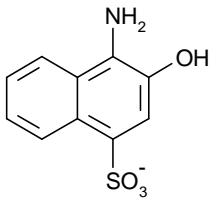
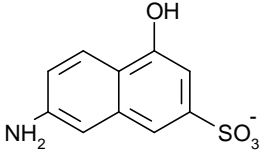
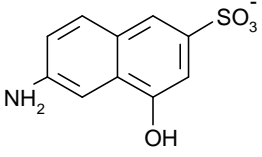
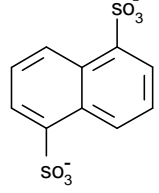
14. L'aplicació de la CZE-ESI-MS va permetre confirmar la presència del 2-NS en una mostra d'aigua de riu què prèviament havia estat analitzada per CZE-DAD sense poder-ne confirmar la presència per l'elució del compost d'interès amb un d'interferent. La CZE-ESI-MS no només va possibilitar identificar aquest compost sinó quantificar-lo en 2.1 mg/l.

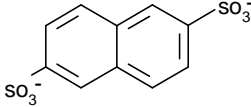
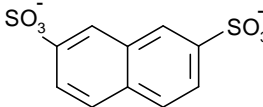
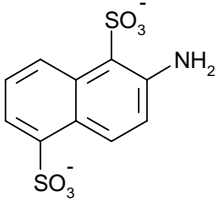
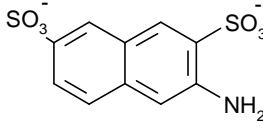
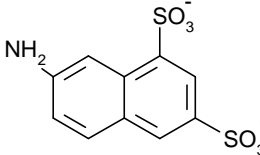
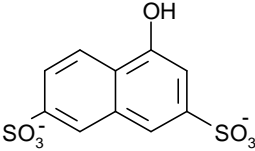
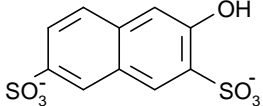
ANNEXOS

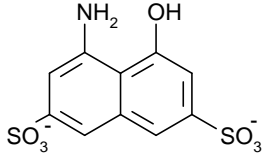
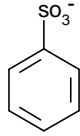
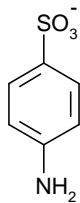
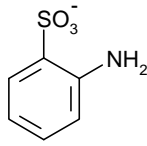
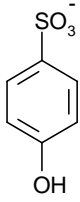
Annex I

Estructura dels compostos estudiats.

Compost	Abreviatura	Estructura
1-naftalensulfonat	1-NS	
2-naftalensulfonat	2-NS	
1-hidroxi-4- naftalensulfonat	1-OH-4-NS	
1-amino-5- naftalensulfonat	1-NH2-5-NS	
2-amino-1- naftalensulfonat	2-NH2-1-NS	
4-amino-1- naftalensulfonat	4-NH2-1-NS	

5-amino-2- naftalensulfonat	5-NH ₂ -2-NS	
8-amino-2- naftalensulfonat	8-NH ₂ -2-NS	
1-amino-2-OH-4- naftalensulfonat	1-NH ₂ -2-OH-4-NS	
6-amino-1-OH-3- naftalensulfonat	6-NH ₂ -1-OH-3-NS	
6-amino-4-OH-2- naftalensulfonat	6-NH ₂ -4-OH-2-NS	
1,5- naftalendisulfonat	1,5-NDS	

2,6- naftalendisulfonat	2,6-NDS	
2,7- naftalendisulfonat	2,7-NDS	
2-amino-1,5- naftalendisulfonat	2-NH2-1,5-NDS	
3-amino-2,7- naftalendisulfonat	3-NH2-2,7-NDS	
7-amino-1,3- naftalendisulfonat	7-NH2-1,3-NDS	
1-hidroxi-3,6- naftalendisulfonat	1-OH-3,6-NDS	
2-hidroxi-3,6- naftalendisulfonat	2-OH-3,6-NDS	

8-amino-1-hidroxi-3,6- naftalendisulfonat	8-NH ₂ -1-OH-3,6-NDS	
benzensulfonat	BZS	
3-amino- benzensulfonat	3-NH ₂ - BZS	
2-amino- benzensulfonat	2-NH ₂ - BZS	
4-hidroxi- benzensulfonat	4-OH- BZS	

Annex II.

Els treballs sorgits de la present tesi, inclosos en els capítols II i III, s'han publicat o estan pendents de publicació en les següents revistes:

M.J. Cugat, F. Borrull, M. Calull, *An overview of electrophoretic methods for the determination of benzene- and naphthalenesulfonates in water samples*, Trends Anal. Chem., 20 (2001) 487-499.

D. Martinez, M.J. Cugat, F. Borrull, M. Calull, *Solid-phase extraction coupling to capillary electrophoresis with emphasis on environmental analysis*, J. Chromatogr. A, 902 (2000) 65-69.

M.J. Cugat, F. Borrull, M. Calull, *Comparative study of capillary zone electrophoresis and micellar electrokinetic chromatography applied to the separation of twelve aromatic sulphonate compounds*, Chromatographia, 46 (1997) 204-208.

M.J. Cugat, F. Borrull, M. Calull, *Evaluation of coelectroosmotic capillary zone electrophoresis for the rapid analysis of twelve aromatic sulphonate compounds*, Chromatographia, 49 (1999) 261-267.

M.J. Cugat, F. Borrull, M. Calull, *Separation of aromatic sulphonate compounds by means of coelectroosmotic micellar electrokinetic chromatography*, Chromatographia, 50 (1999) 229-234.

M.J. Cugat, F. Borrull, M. Calull, *Comparative study of capillary zone electrophoresis and micellar electrokinetic capillary chromatography for the separation of naphthalenedisulfonate isomers*, *Analyst*, 125 (2000) 2236-2240.

M.J. Cugat, F. Borrull, M. Calull, *Large-volume sample stacking used as on-capillary sample enrichment to analyse naphthalene- and benzenesulfonates in real water samples by capillary zone electrophoresis*, *Analyst*, 126 (2001) 1312-1317.

M.J. Cugat, F. Borrull, M. Calull, *Isotachophoretic focusing and mass spectrometry detection as tools of aromatic sulfonates in capillary electrophoresis for improving the determination*, *Electrophoresis*, pendent d'acceptació.